REPORT ON THE

"DETERMINATION OF THE HEAT RESISTANCE OF MICROBIAL ISOLATES FROM THE EASL"

15 April 1967
Task 5.5
JPL CONTRACT 951624

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for

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CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

-NOTE-

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RE-ORDER No. 67-116

CONTENTS

RE-ORDER No. 67-116

ILLUSTRATIONS

TABLES

ABBREVIATIONS

°C	Degree Centigrade
D-Value	The time required to kill, at a given temperature, ninety percent of organisms exposed to the heat cycle
EASL	Experimental Assembly and Sterilization Laboratory
No.	Number
ml	Milliliter
min	Minute
TSA	Trypticase Soy Agar
C	Observed Count
DIL	Power of ten dilution factor
k	Index of experimental conditions
DF	Dilution factor
X	Count correction for dilution ($X = DF \cdot C$)
σ_{C_n}	Expected standard deviation in the n^{th} observed count
σ_n	Expected standard deviation corrected for dilution ($\sigma_n = DF \cdot \sigma_{C_n}$)
$\overline{X}_k$	Average corrected organism count for the k^{th} condition
	Heating time associated with the k^{th} condition, defined so that $T_2 = 0$ corresponds to the time the sample attains temperature
σ_k	Expected error in $\overline{X}_k$
RMS	Root Mean Square

RE-ORDER No. 67-116

I. INTRODUCTION

The purpose of this study was to determine the dry heat resistance of representative bacterial spores isolated from the EASL environment and related environment when plated on a surface. Spores isolated in the EASL environment were assayed for their dry heat resistance at 125°C when plated onto stainless steel strips. The stainless steel surface is considered typical of the surfaces that might be encountered when sterilizing a space capsule. The spore bearing strips were exposed to 125°C for times ranging from 5 to 50 minutes in a laboratory hot air oven and then assayed to determine the number of survivors. The data were then fit, using a weighted least-squares procedure, to an exponential equation and the D value, or time required for a factor of 10 reduction in the number of organisms, determined.

II. TEST PLAN AND PROCEDURES

A. ORGANISMS STUDIED

Listed below are 11 organisms chosen for this study as representative of EASL and related environments isolates; the heat resistances of which are to be determined. The organisms were chosen from those isolated from the EASL and associated areas and identified in Experimental Assembly and Sterilization Laboratory Identification of Microbiological Isolates, 15 April 1967, Task 5.4, JPL Contract 951624.

B. globigii is used as the standard reference culture, and Arthobacter globiformis, while not a spore former, was included in this study because of its demonstrated resistance to die-off in the EASL laminar flow area. This organism forms cystites, and it was desired to determine whether the organism displayed heat resistance characteristics similar to the selected spore formers.

Code No. *	Organism	Remarks
	B. globigii	Standard Reference Culture
M-3	B. subtilis, subspecies A	Representative of isolates from EASL
M-6	B. globigii	Representative of isolates from EASL
E-1	B. pumilis, subspecies A	Representative of isolates from EASL
E-14	B. megaterium, subspecies A	Representative of isolates from EASL
E-15	B. megaterium, subspecies A	Physiologically different from E-14

E-17	Arthobacter globiformis	Forms cystites and showed resistance to die-off in EASL
E-27	B. firmus, subspecies A	Representative of isolates from EASL
SP-51	B. pumilis, subspecies C	Representative of isolates from EASL
SP-53	B. globigii, subspecies B	Representative of isolates from EASL
SP-56	B. megaterium, subspecies B	from EASL

*Reference Experimental Assembly and Sterilization Laboratory Identification of Microbiological Isolates, 15 April 1967, Task 5.4, JPL Contract 951624

B. PREPARATION OF STAINLESS STEEL STRIPS FOR INOCULATION

Stainless steel strips, 1 inch x 1-1/2 inch x 0.02 inch were used,with a hole 6/32-inch diameter at one end to facilitate hanging the strip on the heating rack were used. The strips were prepared for inoculation as follows:

1) Washed in hot detergent solution -- Alconox
2) Rinsed thoroughly in tap water -- at least 5 times
3) Rinsed 3 times in distilled water
4) Rinsed twice in 95 percent ethyl alcohol or isopropyl alcohol
5) Rinsed in ethyl ether
6) Allowed to air dry.
7) The strip surfaces were labelled before sterilization in order to facilitate placement for sonication and to allow for sequential removal of strips from the heating rack.
8) The strips were arranged in glass Petri dishes and sterilized with dry heat at 175^{o}C for 3 hours.

C. PROCEDURE FOR ASSAY

1) Preparation of Spore Suspension

Plate counts of each suspension were made before and after heat shocking of the spore suspensions at 80^{o}C for 20 minutes. The counts were made in order to obtain the number of viable spores in the suspension. The stock suspensions were made in sterile distilled water. Suitable dilution blanks of 1.0-percent peptone water were prepared in Bussey bottles or milk dilution bottles. Sterile peptone water was added aseptically to sterile bottles for the smaller dilution blanks; in other cases the peptone water was placed in the bottles before sterilization. The detailed procedure is outlined below:

a) Dilutions were made to 10^{-7} by steps of 10 from the stock suspension.

b) One ml replicates were plated in triplicate from the 10^{-7}, 10^{-6}, and 10^{-5} dilutions.

c) The dilution bottles were then heat shocked for 20 minutes at 90°C. The heat shock treatment was timed from the time when a thermometer in a bottle containing the same amount of peptone water as the dilution bottles with the test suspension reached 80°C.

d) The heat-shocked diluent was plated in triplicate at the end of the heat shock period as in (b) above.

e) The plates were incubated at 32°C for 72 hours and counted at 24, 48, or 72 hours.

f) The percent of spores and number of spores in the stock suspension were calculated from the colony counts from each dilution series. The average value from the two series was used to establish the number of spores inoculated onto the steel strips.

2. Inoculation of Stainless Steel Strips

a) A 0.2 ml pipette was used to deposit 10^7 to 10^8 spores (0.1 ml) onto the marked side of the strips; the strips remained in sterile Petri dishes during the inoculation.

b) The inoculated strips were placed in a dessicator and dried under vacuum (25 inches below atmospheric) for 16 hours with silica gel as a dessicant. Since the strips were prepared in quantities in 150 mm petri dishes, care was taken to ensure that the strips did not overlap each other in the dishes and that the dishes were not tightly packed in the dessicator.

c) At the end of the drying period, 6 strips were selected as controls for the population determination on the strips. When the strips were heat tested immediately, this assay served as the unheated assay in the test. When strips were prepared in advance and held, control assays were made both at the end of the drying time and before a heat test assay. The length of storage time was noted whenever strips were held before heat testing.

3. Heat Exposure and Survivor Recovery

a) The oven was set at 125°C ± 2.0° and maintained constant at that temperature throughout the study. It was not necessary to open the oven door to insert the stainless steel strips into the oven with the suspension rack used, thereby reducing perturbation of the oven air temperature to a minimum. The rack with the slides was inserted through a small opening in the top of the oven. (See Paragraph c below.)

b) The following heating times were used for each organism. For each heating time a number of different dilutions were plated, as listed below, to obtain colony counts of useful quality.

Time	Dilutions
No Heat	10^{-6}, 10^{-7}, 10^{-8}
Heat-up time	10^{-6}, 10^{-7}, 10^{-8}
Heat-up time + 5 min	10^{-5}, 10^{-6}, 10^{-7}
Heat-up time + 10 min	10^{-2}, 10^{-3}, 10^{-4}
Heat-up time + 20 min	10^{-2}, 10^{-3}, 10^{-4}
Heat-up time + 30 min	undiluted, 10^{-1}, 10^{-2}
Heat-up time + 50 min	undiluted, 10^{-1}, 10^{-2}

c) A rack to hold the test strips in the oven was designed as follows. A 2-inches-long wooden dowel 1/4 inch in diameter was notched at one end to a depth of 5/8 inch, and a hole approximately 1/8 inch drilled through the notched end at 1/4 inch from the notch opening. A stainless steel strip, 1 inch x 1-1/2 inches x 0.02 inch, with a hole at one end (about 1/8-inch diameter), was placed in the notch so that the 3 holes were in alignment. The strip was held in place tightly by Teflon shims and cement. A 1-3/4-inch piece of applicator stick covered with a Teflon tubing sheath was inserted through the holes in the dowel and the metal strip and cemented in place. A thermocouple was wired to the permanently mounted strip to monitor the temperature during each assay. Glass tubing (1/4-inch diameter) rings cut in 3/6-inch lengths served as spacers to maintain the steel test strips in position on the teflon covered bar. The thermometer was removed from its receptacle in the oven roof. The receptacle was then unscrewed and threaded over the free end of the dowel. This allowed the rack to be placed into the oven through the small opening in the roof, thus eliminating excessive heat loss which results when it is necessary to open the oven door for placement of test strips.

d) As discussed in (c) above, a thermocouple was welded to the permanently mounted stainless steel strip on the rack. The thermocouple reading was recorded during each assay in order to provide a record of the time required for the test strips to reach 125°C and to provide a continuous record of the temperature during the time in the oven.

e) For each heating time, six replicate strips were placed on the oven rack; the rack was inserted into the oven until the strips reached 125°C and were kept at 125°C for the specified heating time. The six strips were removed, using sterile forcepts, and each one immediately dropped into 20 ml of sterile, cold (0—10°C), 1-percent peptone water contained in wide-mouth, capped Bussey bottles.

f) The strips were sonicated (with the inoculated side down) for 12 minutes at 25 kc/sec. The bottles were arranged upright, directly contacting the bottom of the sonicator tank in the previously located areas of greatest cavitation, and held in place.

g) Following sonication, the peptone water and the strips were assayed for the number of survivor spores.

4. <u>Assay for Spores in Wash Solution and on Strips</u>

a) By the use of separate sterile forceps for each one, the strips were removed from the bottles and placed on a layer of Trypticase Soy Agar (TSA) in a Petri dish. TSA (50°C) was then poured over the strips until the strips were completely covered in the Petri dish. Each of the bottom and top layers contained approximately 10 ml of TSA.

b) Separate pipettes were used for each dilution in order to make the necessary number of dilutions for the particular test time. One ml aliquots were plated in duplicate from the three highest dilutions prepared.

c) Melted, cooled TSA ($\approx 50°C$) was poured into the aliquots of peptone water, mixed well, and allowed to harden.

d) The plates were aerobically incubated at 32°C for 72 hours, and colonies counted at 24, 48, or 72 hours.

e) The colony count from the test strip was added to the calculated counts from the dilution plates.

D. DATA ANALYSIS

The accumulated count data were analyzed using a program for the Telcomp digital computing system in order to give a weighted least-squares fit of an exponential equation to the data with the associated statistical uncertainties and the D-value.

An individual data point consists of a colony count. Because of counting statistics and limitations inherent in counting densely populated regions, a measurement can have a range of value of only about a factor of 100. That is, counts less than about 20 or greater than about 2,000 are of relatively low reliability. Hence, several different dilutions of the original sample were made in order to extend the practical range of measurement. These dilutions were by a factor of some integer power of 10.

The program developed for this study is designed to

1) correct each observed count for the dilution made;

2) average all the observations for one heating time, weighting each observation according to the expected reliability of the observation.

3) fit an exponential function of time, using a weighted least-squares calculation to the averages of each heating time, thereby determining the associated D-value; and

4) give observed deviations and estimated statistical uncertainties and an indication of the quality of fit.

In averaging, each observation is weighted according to the following relationships:

$$\bar{X} = \frac{\sum_n X_n/\sigma_n^2}{\sum_n 1/\sigma_n^2}$$

X_n = the n^{th} measurement (corrected for dilution)

$\bar{X}$ = the average measurement

σ_n = the expected standard deviation of the n^{th} measurement

It is necessary to make this weighting in order to minimize the expected error in the averages when quantities with varying estimated errors are being averaged.

For any one observed count (C_n), the following estimate of σ_{C_n} was made:

σ_{C_n} = $\bar{C}_n$ (counting statistics) if $\bar{C}_n < 100.$

($\bar{C}_n$ is the average of count taken for any one dilution)

σ_{C_n} = (1/10) $\bar{C}_n$ if $\bar{C}_n > 100$ (general error allowance).

σ_{C_n} = 1 if $\bar{C}_n$ = 0

The standard deviation σ_{C_n} is multiplied by the same factor as C_n when the correction for dilution is made. *(i.e.* $X_n = DF \cdot C_n; \ \sigma_n = DF \cdot \sigma_{C_n}$*)*

The following information is printed out from the program:

1. The ratio of the actual mean square deviation to that estimated:

$$R = \frac{\sum_n \frac{X_n^2}{\sigma_n^2} - \frac{\left(\sum_n \frac{X_n}{\sigma_n^2}\right)^2}{\sum_n \frac{1}{\sigma_n^2}}}{N_k}$$

where N_k is the number of measurements entered for that condition.

2. The average organism count (corrected for dilution):

$$\bar{X}_k = \frac{\sum_n \frac{X_n}{\sigma_n^2}}{\sum_n \frac{1}{\sigma_n^2}}$$

3. The RMS (root mean square) deviation of X_n about $\bar{X}_n$.

$$\sigma_k = \frac{\sqrt{R}}{\sqrt{\sum_n \frac{1}{\sigma_n^2}}}$$

This procedure is repeated for each of the heating times. Then the standard formulas for fitting a straight line are applied to the logarithms of $\bar{X}_k$.

Let

$$L_k = \ln \bar{X}_k$$

$$\sigma_{L_k} = \frac{\sigma_k}{\bar{X}_k}$$

$$a_{11} = \sum_{k=2}^{8} \frac{1}{\sigma_{L_k}^2}$$

$$a_{12} = a_{21} = \sum_{k=2}^{8} \frac{T_k}{\sigma_{L_k}^2}$$

$$a_{22} = \sum_{k=2}^{8} \frac{T_k^2}{\sigma_{L_k}^2}$$

$$b_1 = \sum_{k=2}^{8} \frac{L_k}{\sigma_{L_k}^2}$$

$$b_2 = \sum_{k=2}^{8} \frac{L_k \cdot T_k}{\sigma_{L_k}^2}$$

Let the expected straight line be represented in the form:

Then

$$a_{11}\ F + a_{12}\ G = b_1$$

$$a_{21}\ F + a_{22}\ G = b_2$$

$$F = \frac{b_1 a_{22} - b_2\ a_{12}}{a_{11}\ a_{22} - a_{12}\ a_{21}}$$

$$G = \frac{a_{11}\ b_2 - a_{21}\ b_1}{a_{11}\ a_{22} - a_{12}\ a_{21}}$$

The expected error in F is given by

$$\sigma_F^2 = \frac{a_{22}}{a_{11}\ a_{22} - a_{12}\ a_{21}}.$$

The expected error in G is given by

$$\sigma_G^2 = \frac{a_{11}}{a_{11}\ a_{22} - a_{12}\ a_{21}}.$$

And the correlation between the two errors is

$$\overline{\sigma_F\ \sigma_G} = \frac{-a_{12}}{a_{11}\ a_{22} - a_{12}\ a_{21}}$$

The quantities F, G, and their expected errors are given both in logarithmic and exponential form. The value -2.3026/G is an estimate of D, the time required to reduce the population by a factor of 10, and

$$\sigma_D = 2.3026\ (\sigma_G)/G^2 \ .$$

Then for each heating time, the ratio of the actual square of the deviation of the point from the straight line to the expected error squared is computed, and these numbers are summed for all of the heating times. If only the observed variation in data were causing deviations from the line, these ratios ought to average to about 1, and, hence, the sum should be about the total number of heating times. Actually the sum should be the number of heating times. Actually the sum should be the number of heating times used, minus the number of parameters in the fitted line, which is two in this case. For a number of experiments, this number has a χ^2 distribution that, for 5 degrees of freedom for example, should be less than 9.24 for 90 percent of the time and less than 15.09 for 99 percent of the time. Higher values indicate that there are other factors, in addition to random experimental variation for each heating time, which are causing deviation from the fitted straight lines.

III. RESULTS

The D values obtained from the heat resistant study are summarized below:

D-VALUE SUMMARY

Spore Culture	D ± Standard Deviation (Minutes)
B. globigii (Reference)	12.91 ± 0.08
M-3 B. subtilis, subspecies A	7.51 ± 0.02
M-6 B. globigii	11.96 ± 0.05
E-1 B. pumilus, subspecies A	7.80 ± 0.02
E-14 B. megaterium, subspecies A	4.29 ± 0.02
E-15 B. megaterium, subspecies A	11.14 ± 0.04
E-17 Arthobacter globiformis	*
E-27 B. firmus, subspecies A	7.60 ± 0.02
SP-51 B. Pumilus, subspecies C	6.08 ± 0.03
SP-53 B. globigii, subspecies B	6.16 ± 0.02
SP-56 B. megaterium, subspecies B	7.67 ± 0.04

*No organisms recovered after heat shock, therefore no D-value can be computed

Tables I through X are summaries of the data computed for each spore culture, and Figures 1 through 10 show graphical representations of the data.

IV. DISCUSSION

A. GENERAL

Examination of the D - value data summary shows that the D-values for the spore cultures tested range from 4.29 to 12.91 minutes. On closer scrutiny it can be observed that of the three Bacillus globigii cultures tested, two gave closely similar D-values, while one was less by about one half. Again, large differences were also noted in the D-values of three cultures of Bacillus megaterium. One D-value at 4.29 minutes, another at 7.67 minutes, and another at 11.14 minutes. The two cultures of B. pumilus studied showed similar D - values of 6.08 and 7.80 minutes. It is difficult to explain the differences between D-values of sub-species of the same spore forming organism. When the organisms were identified, differences in the biochemical and physiological characteristics were noted between cultures tentatively identified as the same species. These variations in cellular physiology may also extend to differences in heat resistance properties. The procedure outlined in this report, however, shows that it is possible to reproduce D-values closely, when the same spore culture is used. This procedure is a revision of the one first used in this study, which resulted in questionable data. It was determined that certain changes, if instituted, would result in better, more repeatable data.

For instance, the heat test rack assembly was replaced by a simple assembly that could be placed into the oven through the small thermometer opening at the top of the oven. This obviated the need for opening the door, a cause of considerable heat loss (approximately 5°C at each opening). The other major problem was the total immersion of the Bussey bottles for sonication to remove the spores from the strips in the bottles. The problem here was serious contamination that ruined many tests either by altering the count or by total overgrowth of the plates. This was corrected by using stainless steel slides, 1 inch x 1-1/2 inch, which could lay flat at the bottom of the Bussey bottle for sonication. The bottles could then be sonicated in an upright position, thereby avoiding this type of contamination.

The revised procedures are the ones described in this report, but it is felt that, although the removal by sonication of the heat exposed spore cells from the strips is at this time the best way of removal, total removal is not always obtained, and some further experimentation is necessary to determine correction of the situation.

B. DISCUSSION OF THE INDIVIDUAL TABLES AND FIGURES

A satisfactory set of data were obtained for the standard Bacillus globigii spore culture. The number of spores surviving the 3-minute increase in temperature from 25°C to 125°C was 93.4 percent. The data points shown on Figure 1 appear to fit the line described by the computer print-out. The points obtained for 5 and 30 minutes are equidistant below the line drawn, but can still be considered good points, since they are within one log value. The D-value was found to be 12.91 minutes.

TABLE I

HEAT RESISTANCE: BACILLUS GLOBIGII

Heat Treatment (minutes)	Average	RMS Error	Chi Square
Control (no heat)	1.314×10^{8}	2.171×10^{6}	2.297×10^{2}
Come up time to 125°C	8.771×10^{6}	3.947×10^{5}	
Come up time + 5	1.460×10^{6}	1.183×10^{5}	
Come up time +10	9.043×10^{5}	5.336×10^{4}	
Come up time +20	2.629×10^{5}	6.317×10^{3}	
Come up time +30	1.507×10^{4}	1.587×10^{3}	

TABLE II

HEAT RESISTANCE: BACILLUS SUBTILIS (M-3)

Heat Treatment (minutes)	Average	RMS Error	Chi Square
Control (no heat)	6.426×10^{7}	3.187×10^{6}	7.25×10^{2}
Come up time to 125°C (3 min)	4.718×10^{7}	1.327×10^{6}	
Come up time +5	---	---	
Come up time +10	7.222×10^{5}	3.276×10^{4}	
Come up time +20	8.000×10^{4}	5.184×10^{3}	
Come up time +30	2.187×10^{3}	1.278×10^{2}	
Come up time +50	2.976×10^{2}	4.847×10^{1}	

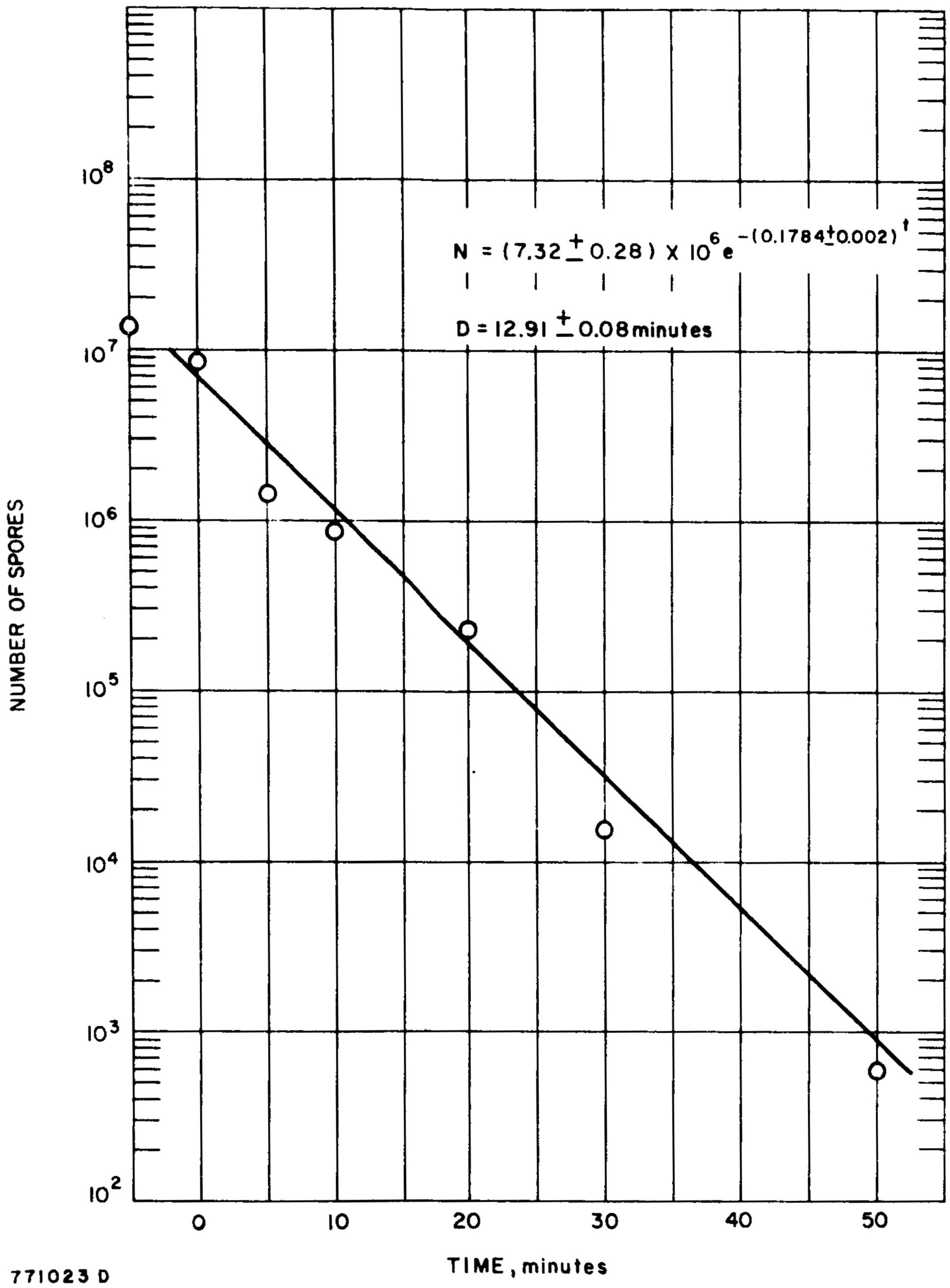

Figure 1. HEAT RESISTANCE: BACILLUS GLOBIGII

RE-ORDER No. 67-116

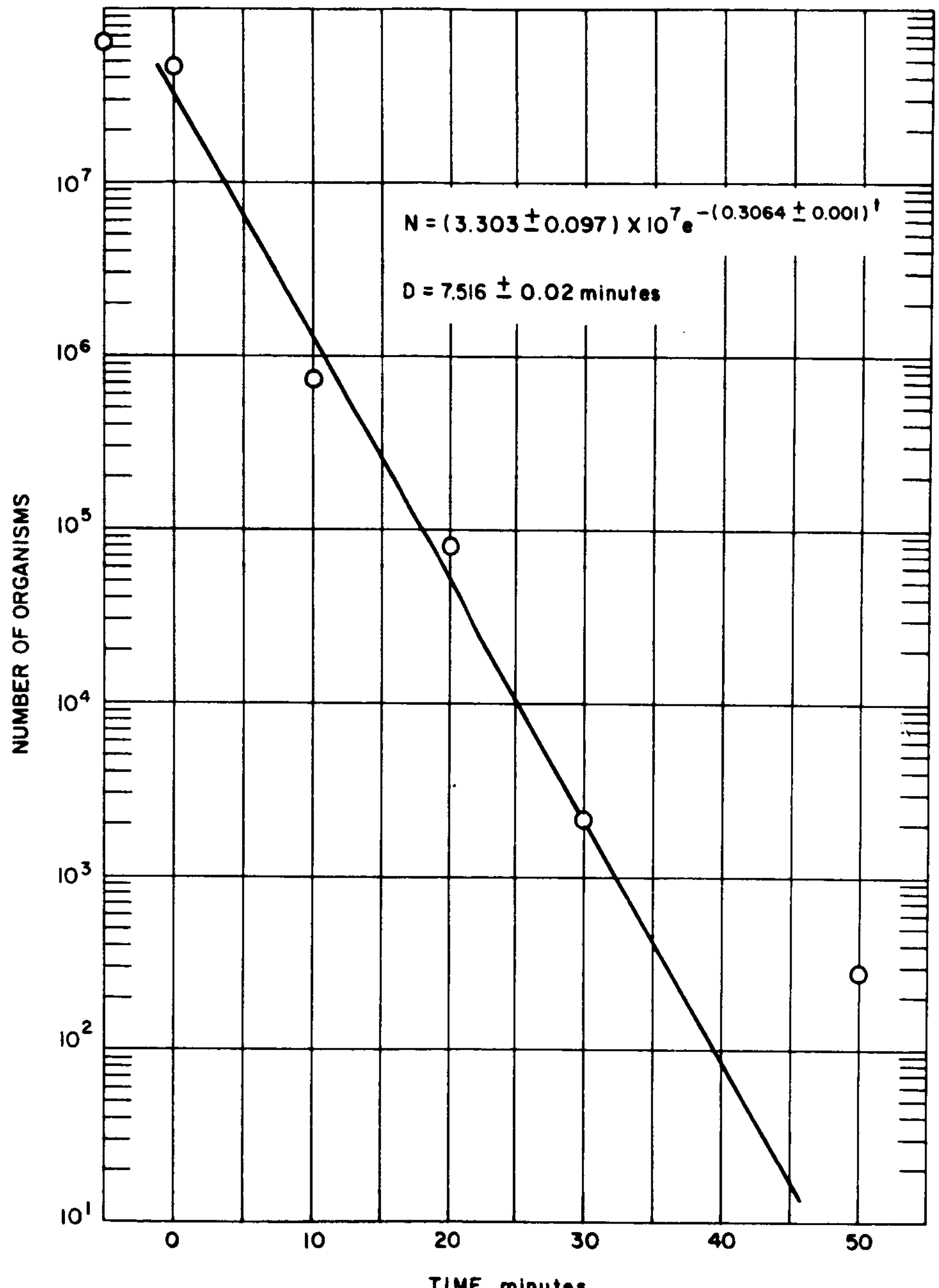

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Figure 2. HEAT RESISTANCE: BACILLUS SUBTILIS (M-3)

TABLE III

HEAT RESISTANCE: BACILLUS GLOBIGII (M-6)

Heat Treatment (minutes)	Average	RMS Error	Chi Square
Control (no heat	1.662×10^7	1.302×10^6	4.830×10^2
Come up to 125°C (3 min)	6.414×10^6	1.376×10^5	
Come up to +5	3.667×10^6	7.896×10^4	
Come up to +10	1.188×10^6	8.006×10^4	
Come up to +20	6.148×10^4	3.827×10^3	
Come up to +30	1.708×10^4	1.536×10^3	
Come up to +50	1.083×10^3	1.199×10^2	

TABLE IV

HEAT RESISTANCE: BACILLUS PUMILUS (E-1)

Heat Treatment (min)	Average	RMS Error	Chi Square
Control (no heat)	1.740×10^8	1.255×10^7	6.691×10^1
Come up time to 125°C (3 min)	1.197×10^8	3.672×10^6	
Come up time +5	2.246×10^7	1.664×10^6	
Come up time +10	9.000×10^6	8.101×10^5	
Come up time +20	1.000×10^6	1.764×10^5	
Come up time +30	1.170×10^4	1.395×10^3	

RE-ORDER No. 67-116

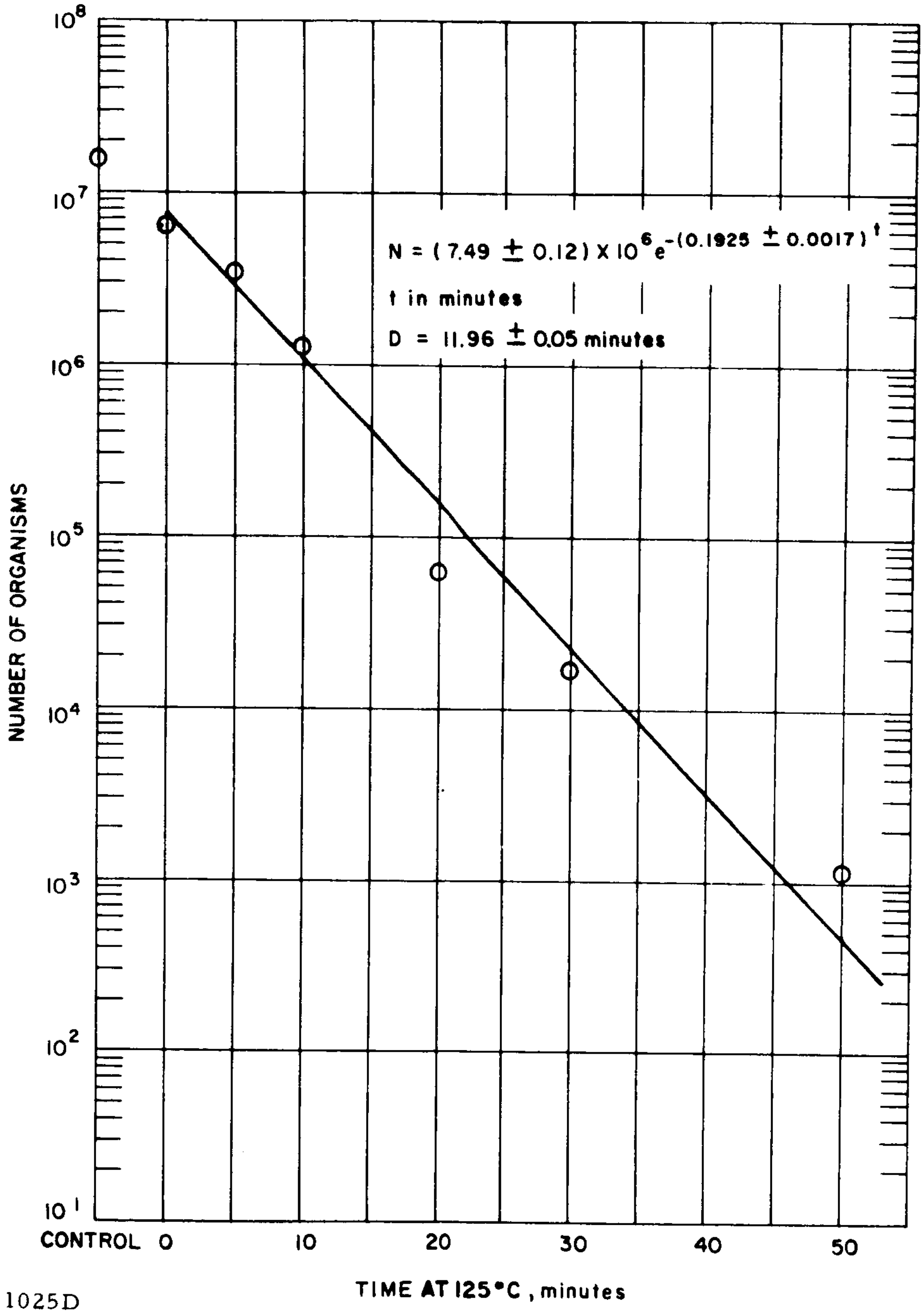

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Figure 3. HEAT RESISTANCE: BACILLUS GLOBIGII (M-6)

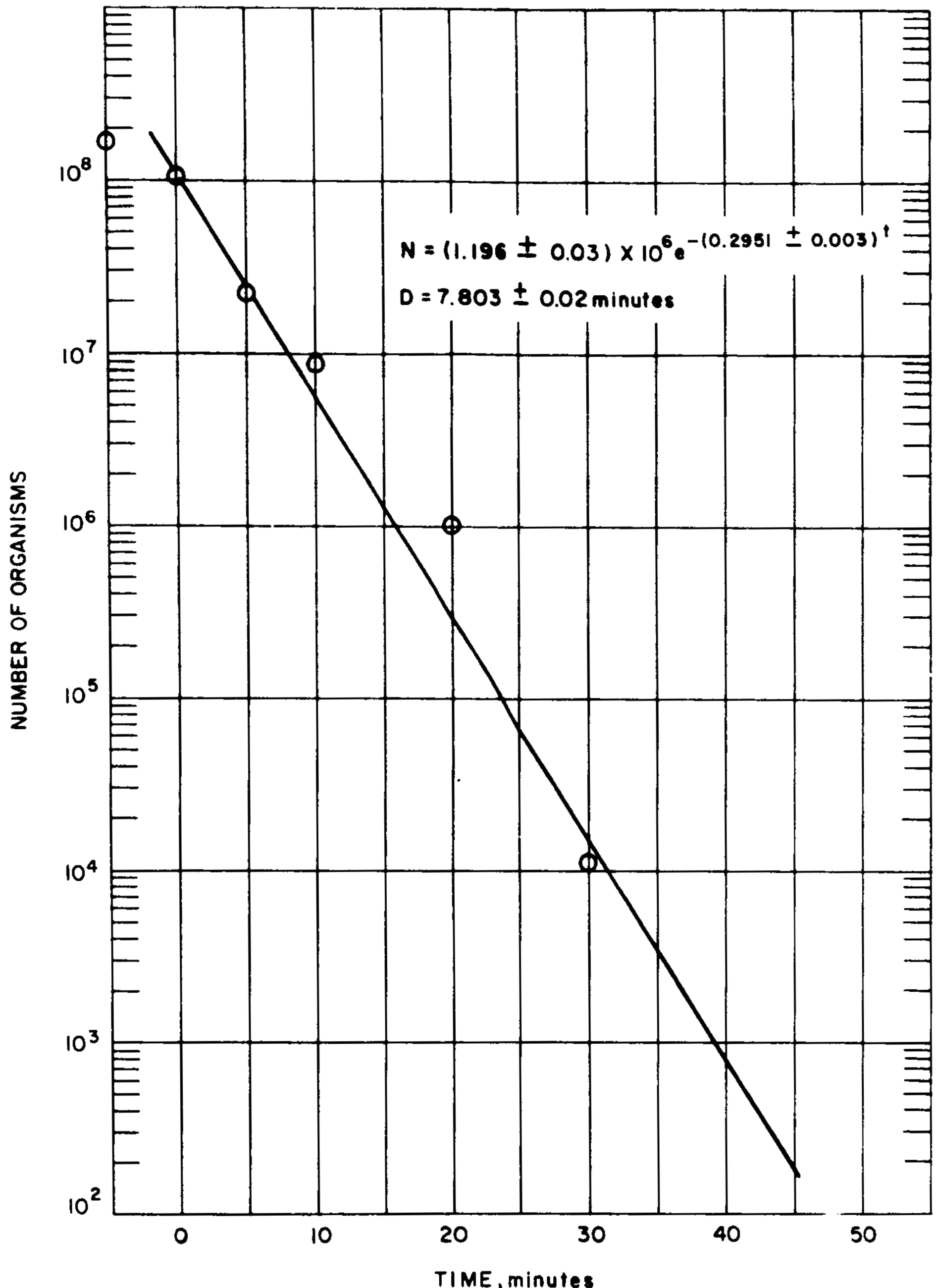

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Figure 4. HEAT RESISTANCE: BACILLUS PUMILUS (E-1)

TABLE V

HEAT RESISTANCE: BACILLUS MEGATERIUM (E-14)

Heat Treatment (min)	Average	RMS Error	Chi Square
Control (no heat)	6.804×10^7	3.298×10^6	5.969×10^2
Come up time to 125°C (3 min)	5.205×10^7	1.820×10^6	
Come up time +5	2.035×10^7	1.945×10^6	
Come up time +10	1.746×10^5	5.970×10^3	
Come up time +20	7.444×10^3	1.101×10^3	
Come up time +30	2.000×10^1	0	

TABLE VI

HEAT RESISTANCE: BACILLUS MEGATERIUM (E-15)

Heat Treatment (Minutes)	Average	RMS Error	Chi Square
Control (no heat)	9.835×10^6	5.016×10^5	1.072×10^3
Come up time to 125°C (3 min)	8.194×10^6	4.258×10^5	
Come up time +5	2.698×10^6	4.677×10^5	
Come up time +10	1.479×10^5	1.102×10^4	
Come up time +20	2.857×10^4	1.997×10^3	
Come up time +30	2.554×10^3	2.015×10^2	
Come up time +50	5.116×10^2	4.381×10^1	

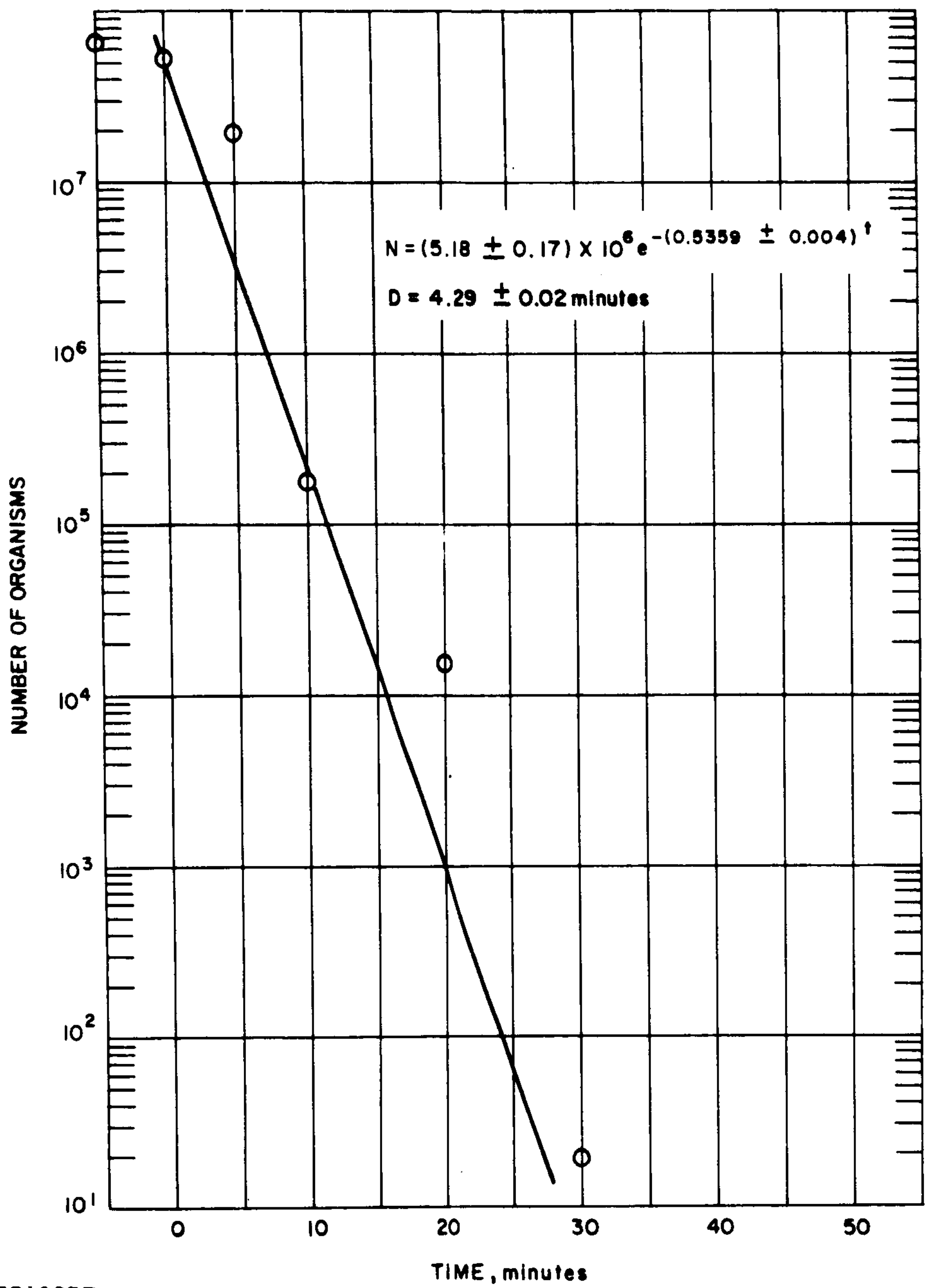

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Figure 5. HEAT RESISTANCE: BACILLUS MEGATERIUM (E-14)

RE-ORDER No. 67-116

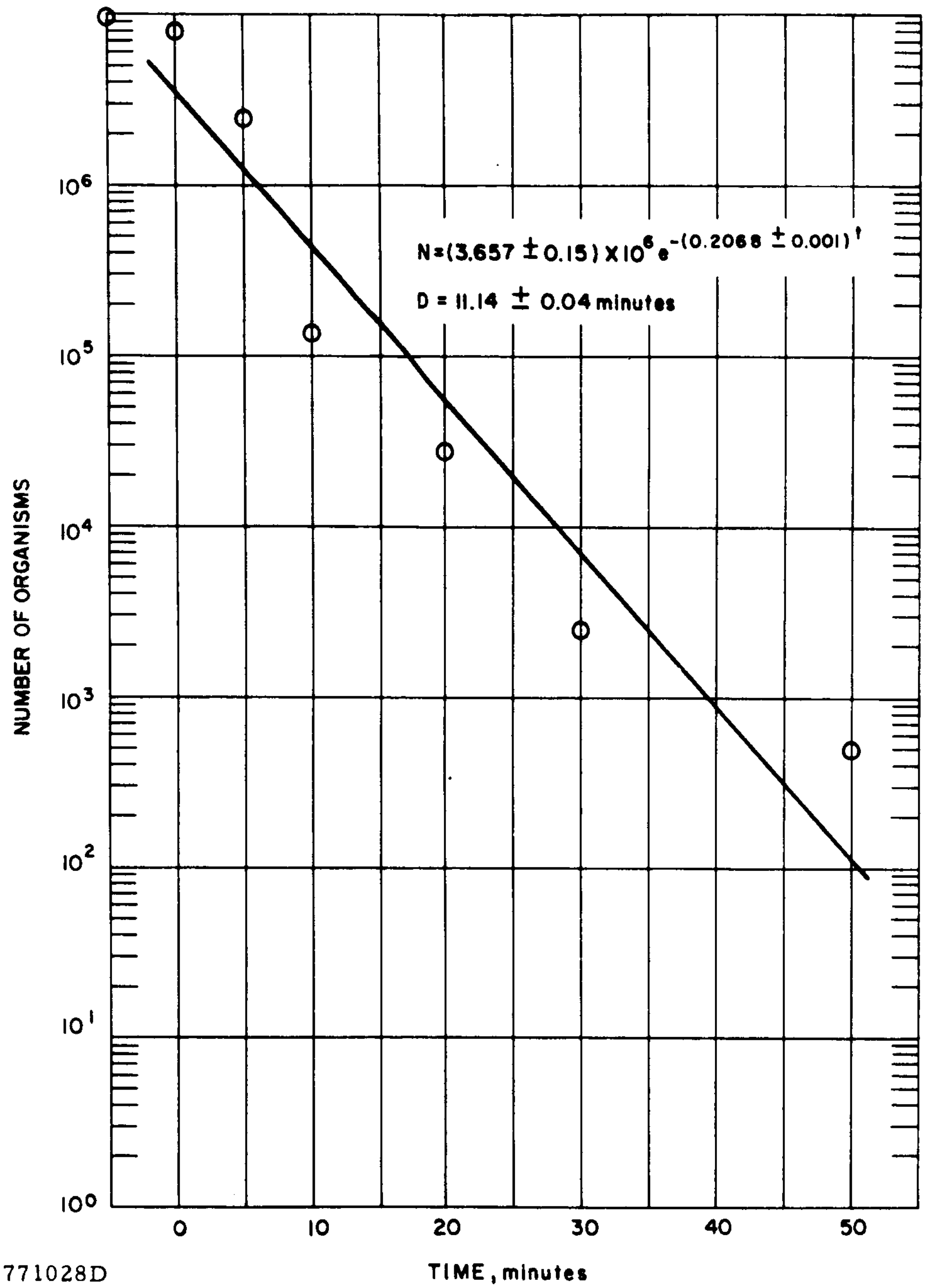

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Figure 6. HEAT RESISTANCE: BACILLUS MEGATERIUM (E-15)

RE-ORDER No. 67-116

TABLE VII

HEAT RESISTANCE: BACILLUS FIRMUS (E-27)

Heat Treatment (minutes)	Average	RMS Error	Chi Square
Control (no heat)	2.311×10^8		2.221×10^3
Come up time to 125°C (3 min)	1.690×10^8	6.108×10^6	
Come up time +5	6.383×10^7	3.328×10^6	
Come up time +10	1.970×10^6	1.513×10^5	
Come up time +20	5.531×10^4	3.039×10^3	
Come up time +30	1.049×10^4	4.140×10^2	
Come up time +50	3.93×10^2	2.80×10^1	

TABLE VIII

HEAT RESISTANCE: BACILLUS PUMILUS (SP-51)

Heat Treatment (min)	Average	RMS Error	Chi Square
Control (no heat)	2.450×10^7	8.491×10^5	7.951×10^1
Come up time to 125°C (3 min)	2.266×10^7	1.757×10^6	
Come up time +5	1.317×10^7	1.853×10^6	
Come up time +10	4.547×10^5	8.724×10^4	
Come up time +20	1.622×10^4	1.261×10^3	
Come up time +30	2.644×10^2	3.650×10^1	

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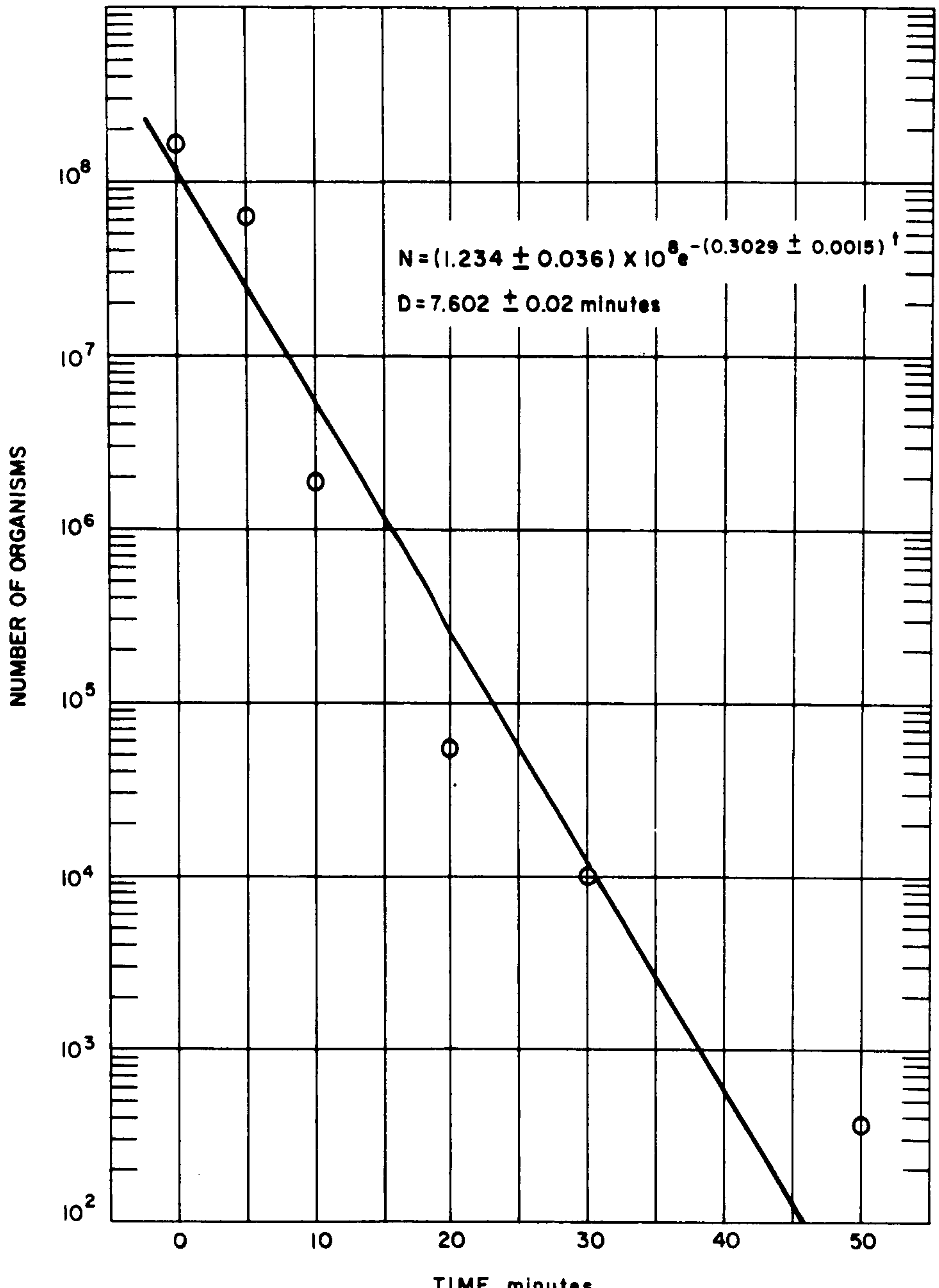

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Figure 7. HEAT RESISTANCE: BACILLUS FIRMUS (E-27)

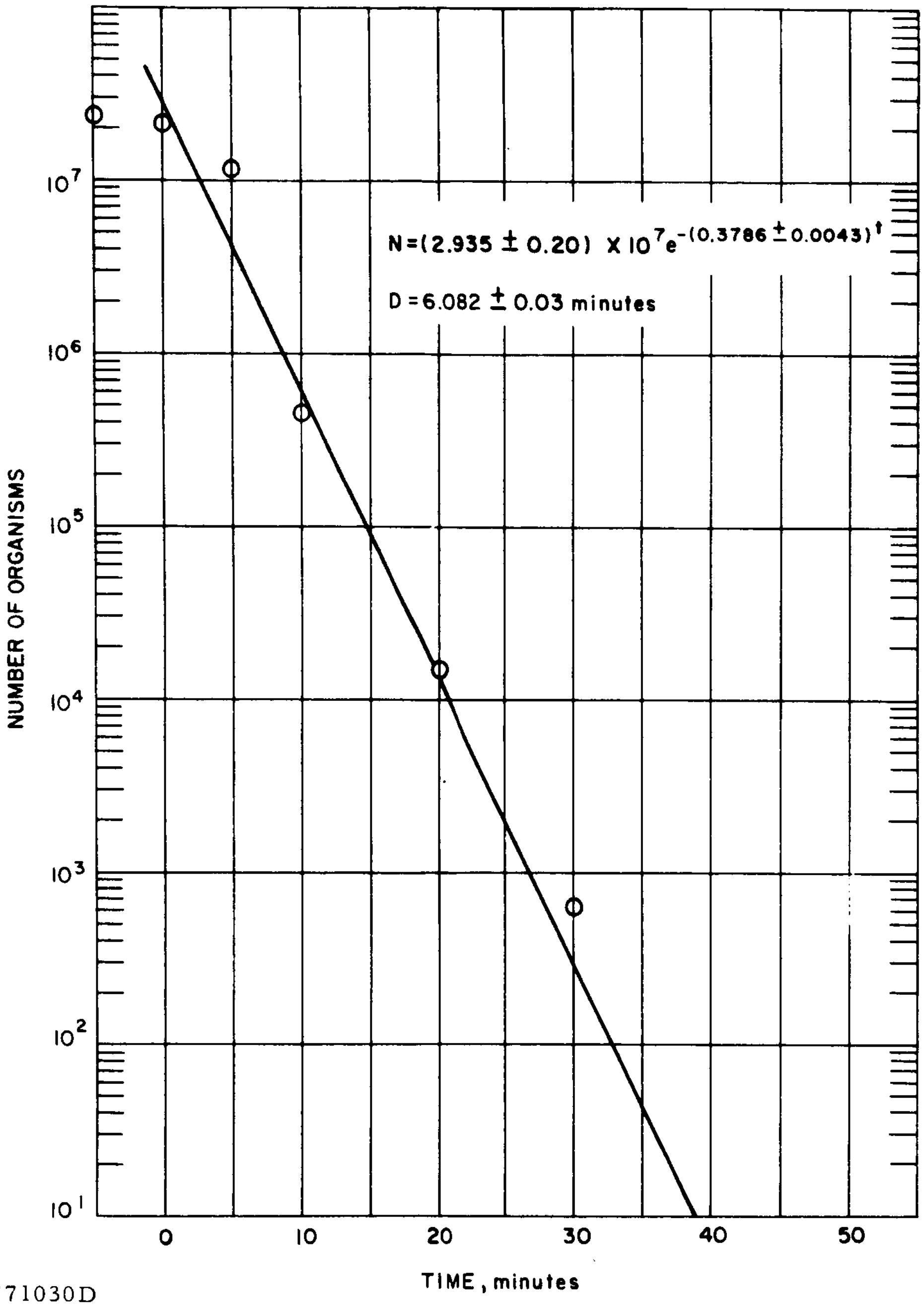

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Figure 8. HEAT RESISTANCE: BACILLUS PUMILUS (SP-51)

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TABLE IX

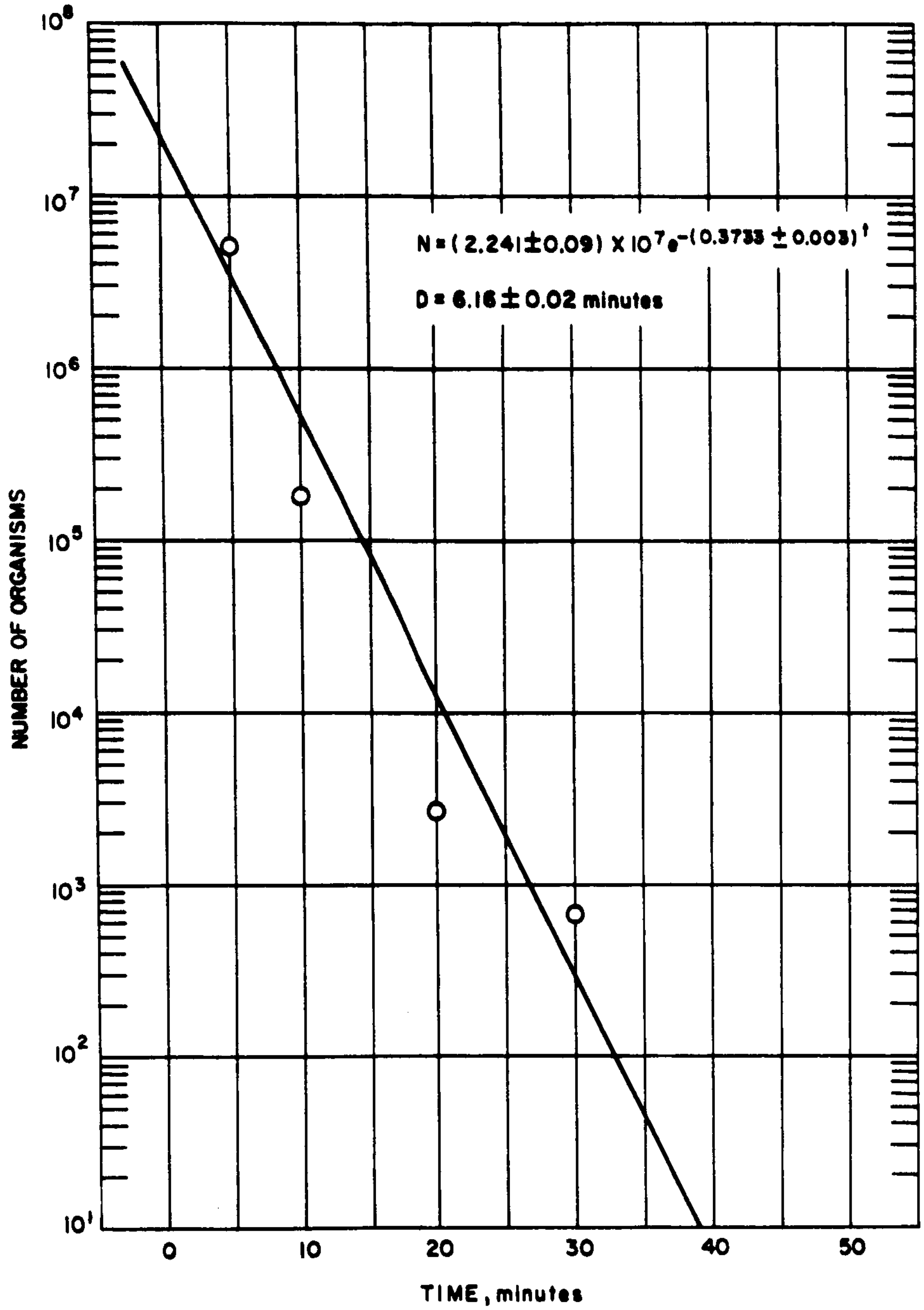
HEAT RESISTANCE: BACILLUS GLOBIGII (SP-53)

Heat Treatment (minutes)	Average	RMS Error	Cni Square
Control (no heat)	1.548×10^8	6.317×10^6	1.638×10^3
Come up time to 125°C (3 min)	1.569×10^8	1.294×10^7	
Come up time +5	5.076×10^6	2.694×10^5	
Come up time +10	1.812×10^5	8.516×10^3	
Come up time +20	2.588×10^3	2.680×10^2	
Come up time +30	6.520×10^2	4.100×10^1	

TABLE X

HEAT RESISTANCE: BACILLUS MEGATERIUM (SP-56)

Heat Treatment (min)	Average	RMS Error	Chi Square
Control (no heat)	1.019×10^8	3.267×10^6	3.230×10^2
Come up time to 125°C (3 min)	2.080×10^8	2.063×10^7	
Come up time +5	2.175×10^7	3.87×10^6	
Come up time +10	1.489×10^6	9.605×10^4	
Come up time +20	1.925×10^5	1.829×10^4	
Come up time +30	7.268×10^3	1.052×10^3	
Come up time +50	1.500×10^2	9.81	



771031D Figure 9. HEAT RESISTANCE: BACILLUS GLOBIGII (SP-53)

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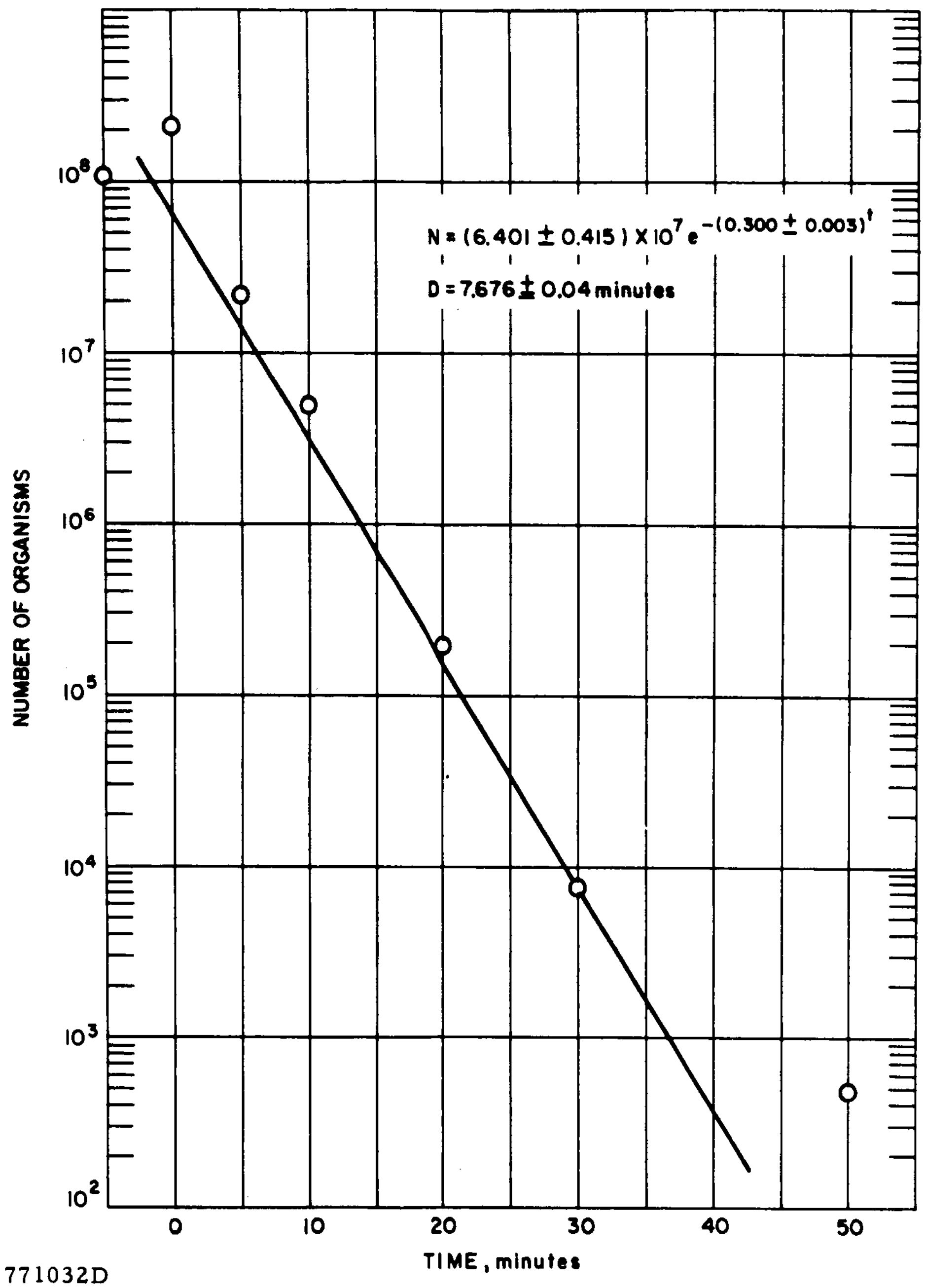

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Figure 10. HEAT RESISTANCE: BACILLUS MEGATERIUM (SP-56)

The data for the Bacillus subtilis (M3) shown in Table II are statistically significant from when the test strips reach 125 °C, through 30-minute of heat exposure. The data at the 50-minute exposure, however, were found to be divergent and does not fit the curve described. The D-value for this culture was found to be 7.51 minutes.

Culture M-6 (Table III and Figure 3), which is another culture of Bacillus globigii, was found almost identical in its heat resistance to the reference culture of Bacillus globigii. The D-value was found to be 11.96 minutes. All points except those of 20 minutes and 50 minutes were close fits to the line described by the print-out. These, however, were both above and below the line in Figure 3 and, therefore, are not considered excessively divergent.

The data for Figure 4 are valid, and the line drawn for B. pumilus shows a fit. The D-value was found to be 7.80 minutes.

Culture E-14, Bacillus megaterium, shows a set of data points (Figure 5) that would indicate the culture is not very resistant to heat at 125 °C for any considerable length of time. In fact, it was found to have a D-value of only 4.29 minutes. The data points at 5 and 20 minutes were above the line described, but the slope between them was as shown by the curve in Figure 5.

The data for another physiologically different culture of Bacillus megaterium is shown inTable VI. Figure 6 shows that the points for the zero time, 5 minute, and 50 minute plots assays fall along the same line, while those of the 10-, 20-, and 30- minute assays fall along another line parallel to the first. It is possible that the sonication removal of the cells from the strips was incomplete during one series of times. The D-values of 11.14 minutes from the print-out results in a line that falls equidistant among the six points.

Examination of the data in Table VII and the points in Figure 7 shows that Bacillus firmus is not very resistant to 125 °C, with a D-value of 7.60 minutes. Only two of the six points in Figure 7 fall along the line described from the printout.

Table VIII and Figure 8 show valid data points for SP-51, Bacillus pumilus, (subspecies C) with a D-value of 6.08 minutes. Four of the five points fall along the line curve. At the 5 minute point, however, there seems to be the characteristic hump that appeared in all of the other line graphs. With a D-value of 6.08 minutes, the SP-51 organism does not appear to be very resistant to the 125°C temperature.

Figure 9 shows a set of points for B. globigii (SP-53). The 10- and 20-minute plots are below the line and appear to be due to a possible problem in the sonication removal of the spores from the strips during the recovery process. Unless Bacillus globigii, subspecies B is radically different from the standard reference Bacillus globigii, the D-value of 6.16 minutes for SP-53 is very low when compared with those in Figures 1 and 3.

RE-ORDER No. 67-116

In Figure 10 (SP-56), Bacillus megaterium, (subspecies B), the 0- and 50-minute points are irregular. The zero point increases in number over the no heat control, even after heating. All of the other points, however fall in good order along the line described from the printout. A D-value of 7.67 minutes was obtained for this culture.

V. CONCLUSION

These data indicate that certain subspecies or varieties of B. globigii are the most resistant to dry heat of the cultures examined. Of the eleven micro-organisms examined Arthrobacter globiformis (E-17) was the most heat sensitive. The cystite forms of thin organism did not survive the heat treatment of 80°C for 20 minutes, although associated studies have revealed them more heat resistant to dessication than vegetative cells (task 5.3, JPL contract 951624). B. megaterium subspecies A (E-14) was found to be the most susceptable to heat of the three spores examined. The most heat resistant spores are approximetely three times more resistant to heat than the least resistant spores. It must be remembered that the reported D-values are only representative of organisms exposed to dry heat (125°C) on stainless steel surfaces. Further evaluation is necessary before it can be stated that the reported D-values are representative of the situation that may be seen by microorganisms on the surface of a spacecraft during dry heat exposure.

REFERENCES

Standard Procedures for the Microbiological Examination of Spacecraft Hardware, 1 June 1966, NASA, Washington, D.C.